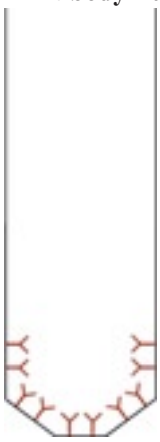
Romer Accutox Method for DON

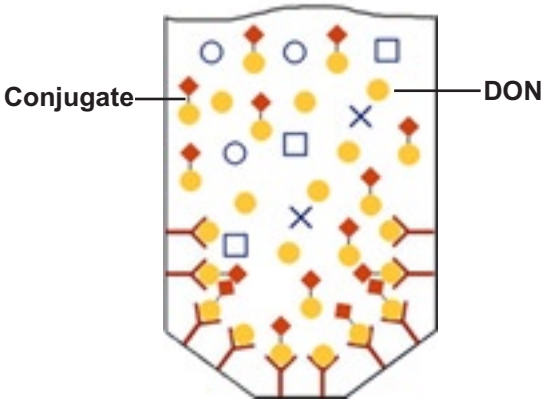
Overview. Romer Lab's "AccuTox" test for deoxynivalenol is an ELISA method. Antibodies specific for a mycotoxin are adhered to the bottom of a tube.

Figure 12. Romer Antibody Tube.



A sample to be tested for DON is ground, extracted with distilled water, and filtered. A solution of DON, chemically conjugated to an enzyme, is provided with the kit. The filtered extract is mixed with an equal amount of the DON-enzyme conjugate in an antibody tube.

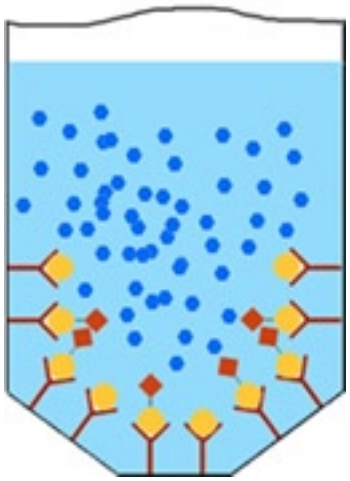
Figure 13. Free toxin and conjugate compete for binding sites in microwell containing antibodies.



After the toxin and conjugate have had time to attach to the antibodies, the remaining solution is discarded and the tube rinsed with Wash Solution.

After tapping the tubes on a paper towel to remove the water, substrate solution is added. The conjugated enzyme present causes the substrate to turn blue. The more conjugate, the more intense the color.

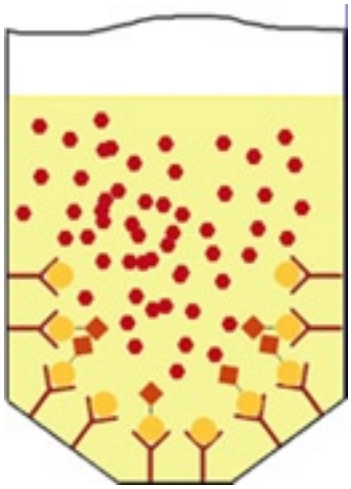
Figure 14. After washing. substrate is added which reacts with the conjugated enzyme to form a blue color.



After allowing the color change to develop for five minutes, "Stop Solution" is added. This stops the reaction and changes the color of the solution to yellow.

Quantitative measurements are obtained by measuring the intensity of the color with a Hach spectrophotometer. Because samples with high DON levels will result in less binding of the conjugate, positive samples will be lighter color.

Figure 15. The solution turns yellow when the stop solution is added.



AccuTox Procedures. The Romer Accutox DON test is provided as a kit containing all required reagents, controls and antibody tubes

NOTE: When not in use, kits should be stored in the dark at refrigeration temperatures. Prior to use, all necessary kit components must be equilibrated to room temperature (approximately 1 hour). The use of cold reagents could adversely affect the color development of the ELISA test.

The kit components are as follows:

-Antibody coated tubes in zip-lock bag
-Substrate.
-Enzyme Conjugate.
-Zero Calibrator Standard
-Control Solution
-Stop Solution.
-Wash solution. Store at room temperature

TIP: Swirl, don't shake all reagents prior to use so as to mix them but not cause them to foam, which could cause pipetting errors later.

Photo 15. Accutox kit components.

Step 1: Thoroughly mix the ground sample and weigh out 50 grams. Place the ground sample in an 18 ounce nasco whirlpack bag or clean plastic or glass container.

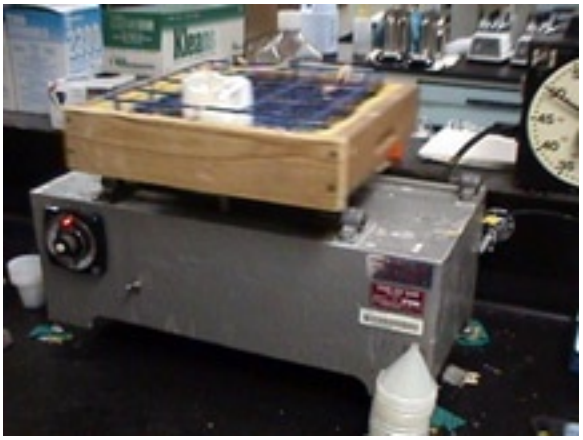
Photo 16. Weighing 50 gram portion.

Step 2: Add 250 ml of distilled or deionized water. **Do not use tap water!** The pH of tap water may adversely effect results.

Step 3: Shake mechanically or by hand for 3 minutes. Let the material stand for 1-2 minutes to let some of the sample settle.

Step 4: Place a sheet of filter paper (Whatman #1 folded, S&S 24-cm pleated, or equivalent) into a clean funnel mounted over a test tube or collection beaker.

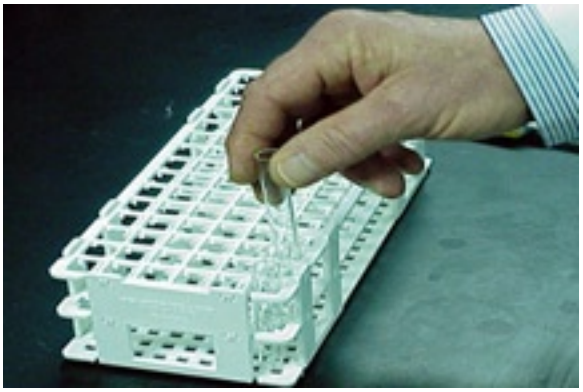
Photo 17. Grain sizer used to shake DON samples.



After much of the sample slurry has settled to the bottom of the bag, pour at least 15 mL of the extract through the filter paper.

Step 5: Place the appropriate number of labelled anti-body coated tubes into gripper tube rack.

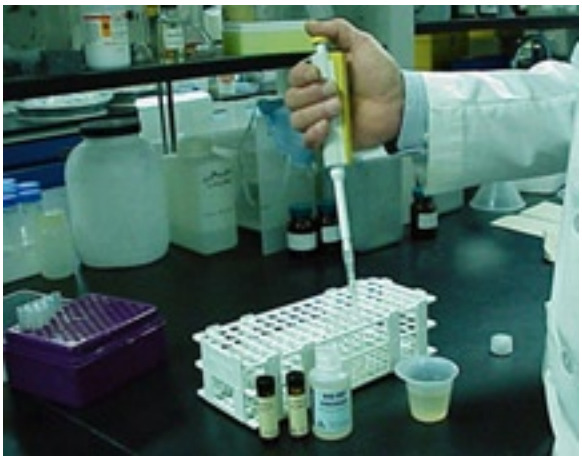
Photo 18. Place tubes in gripper rack.



NOTE: Do not exceed 30 tubes per run. Be sure to re-seal unused tubes in zip-lock bag with desiccant.

Step 6: Pipet 0.5 ml of zero calibrator into appropriate antibody-coated tube and discard tip. repeat this step for the control and filtered sample extracts .

Photo 19. Adding zero calibrator, control, and sample extracts to tubes.



Step 7: Pipet 0.5 ml of Enzyme Conjugate into each tube and incubate for 15 minutes.

NOTE: Start timing for 15 minutes as soon as conjugate has been added to first tube.

Photo 20. Incubate for 15 minutes.

TIP: Prime pipette tips before dispensing conjugate. To prime a tip, draw up a pre-measured amount of the reagent to be dispensed and discharge it back into the same container. Priming coats the inside of the pipette tip so that the volume dispensed will be identical during repeated use of the same tip.

Step 8: Mix contents of tubes by shaking rack vigorously for 5 seconds.

During this incubation, the conjugated DON and any free DON from the sample or standard will compete for binding sites on the antibody tube. The more free DON in solution, the less conjugate will be bound to the antibody well.

Step 9: At the completion of the 15 minute incubation dump contents of tubes into appropriate waste container. (e.g. sink, bucket, etc.). Fill tubes with Wash Solution and dump wash. Repeat three times for a total of four washes.

Photo 21. Wash tubes four times.

NOTE: It is very important not to under-wash tubes. Overwashing will not affect the test.

Step 10: Following last wash, tap inverted tubes onto absorbant paper several times to remove all wash solution.

NOTE: It is very important to rid tubes of as much wash as possible.

Step 11: Pipet 0.5 ml of substrate into each tube and incubate or 5 minutes.

Photo 22. Tapping tubes on paper towel to remove wash solution.

NOTE: Start timing for 5 minutes as soon as substrate has been added to first tube.

NOTE: Solutions should all turn blue after substrate has been added.

Photo 23. Five minute incubation. Solutions turn blue after substrate is added.

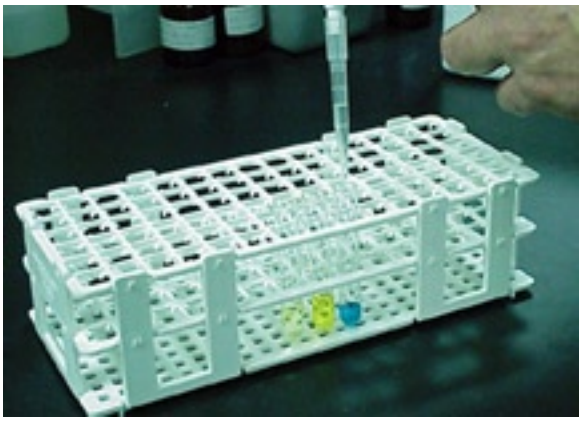
Step 12: Mix contents of tubes by shaking rack vigorously for 5 seconds.

Step 13: At the completion of the 5 minute incubation, pipet 0.5 ml of Stop Solution into each test tube.

Step 14: Mix contents of tubes by shaking rack vigorously for 5 seconds.

NOTE: Solutions should all turn yellow after adding Stop Solution.

Photo 24. Solutions turn yellow when the Stop solution is added.



Step 15: With the spectrophotometer set at 450 nm, blank with a clean unscratched test tube filled with fresh distilled or deionized water.

Step 16: Dry each tube with a paper towel before inserting into spectrophotometer. read and record absorbances of the calibrator, control, and samples.

Photo 25. Hach Spectrophotometer.

Step 17: Calculate results using log/logit data computer program with the factory calibration included with the kit.

NOTE: The control must fall within the acceptable range listed on the control vial. If not, troubleshoot and repeat the run. Call Romer Labs Technical Services (1-800-769-1380) for troubleshooting assistance.